Variation, heredity and selection of effective ingredients in *Magnolis officinalis* of different provenances

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Abstract: Bark samples of *Magnolis officinalis* were collected from a 7-year-old trial plantation with 13 provenances in Jingning County of Zhejiang Province on June 25, 2000. The contents of magnolol and honokiol of *M. officinalis* were analyzed by the method of HPLC (High Performance Liquid Chromatogram). The results showed that such qualitative traits as the content of magnolol, content of honokiol, total content of key phenols, and the ratio of magnolo to honokiol differ significantly between the provenances. The provenances with a sharpened leaf tip from the western part of Hubei Province has a highest content of phenols, and that with a concave leaf tip from the Lushan Mountain has a lowest content of phenols. All these four qualitative traits were genetically controlled, with a heritability between 0.8342 and 0.9871 in terms of provenance. In addition, both longitudinal and latitudinal geographical variations could be found, with longitudinal variations being dominant. As a result, 3 superior provenances from Wufeng, Enshi and Hefeng of the western part of Hubei as well as 10 high-quality individuals were selected.

Keywords: *Magnolia officinalis*; Provenance trial; Magnolol; Honokiol; Geographical variation; Provenance selection

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Introduction

Taxonomically speaking, *Magnolia officinalis* for medicinal purposes includes two subspecies, *Magnolia officinalis* Rehd. et Wils. subsp. *officinalis*, and *M. officinalis* subsp. *biloba* (Rehd. et Wils.) Law and those intermediate between these two subspecies, all of which are peculiar to China and list in the second category of protecting Chinese medicinal plants. *M. officinalis* mainly distributed on hilly land of Hubei, Sichuan, Gansu, Shaanxi, Hunan, Anhui, Zhejiang, Jiangxi Fujian, Guangxi Provinces, etc. at the elevation of 200-3 000 m. As exploitation and other human activities have led to the shrinking of the natural resources, so plantation establishment has attracted the attention of the government and common people of the producing areas.

The effective ingredients of *M. officinalis* are mainly phenols and alkaloids, of which magnolol and its isomer honokiol absolutely dominate. It has been testified that this two ingredients have a strong effect on antiphlogistic and sterilization in pharmacology (Lu 1989). The bark quality is mainly shown by the contents of these two ingredients. It's recorded in *China Pharmacopoeia* that the bark of *M. officinalis* produced in different areas and had different quality, as the high quality bark mainly produced in Hubei and Sichuan, followed by those in Fujian and Zhejiang (Pharmacopoeia Commission 2000). In traditional Chinese medicine, great importance is attached to the genuineness of the medicine. In a traditional opinion, quality of the medicine is mainly influenced by environmental conditions of the producing area, such as climate and soil. Now owing to no systematic studies on intraspecific geographical variations in quality, many studies on quality of *M. officinalis* mainly concentrated on tree age, appearance traits, anatomic characteristics of the bark, etc. (Zhao *et al.* 1992; Si *et al.* 1998; Li *et al.* 1994; Si *et al.* 1994). Based on systematic biological studies on this species, the seed of *M. officinalis* from 13 provenances in main producing areas were collected, and was trial plantation in Jingning County of Zhejiang Province. Variations in the content of phenols and its heritability for a 7 years old plantation of provenance are studied. This study will lay a foundation for the selection of superior provenance and individuals.

Materials and methods

Collection and treatment of sample

Seeds of *M. officinalis* from 13 provenances in main producing areas (see Table 1) were collected in 1992, and seeded on the Caoyutang Forest Farm of Jingning County, Zhejiang Province in 1994. With a soil of zheltozem, the trial plantation is located in hilly land at latitude 29°51'N and longitude 119°41'E and at an elevation between 1 000 and 1 100 m. In each quadrat, there were 3 replications, and each single-row plot contained 15 individual trees, with Jingning 1 and Jingning 2 as a control (Si *et al.* 1998). Bark samples were collected from the trunk at 95-105 cm on

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June 15th of 2000, each provenance sampled from 5 individual trees respectively. Totally 195 samples were analyzed. When samples were collected, such traits as DBH, tree height, bark thickness and leaf shape were recorded. All the samples were dried naturally in the shade.

Analyses of the contents of magnolol and honokiol

The contents of both magnolol and honokiol were analyzed by the method of HPLC (High Performance Liquid Chromatogram). This instruments included Waters 600 controller and Waters 486 UV detector. A wavelength of 300 nm was adopted. The HPLC-grade methanol was produced by Shanghai Ludu Industrial Co.. The standard samples of both magnolol and honokiol were produced by China Identification Institute of Bioproducts. All the data were processed with the help of area normalization of external standard method (Song 1990). Oiliness was determined according to whether there was oil to be found in the sample, when the sample was grinded, expressed as 1 and 0.

Procession of data

For each trait, variance analysis and analyses of genetic correlation and phenotypic correlation were conducted according to the method of Ma Yuhua (1978).

Results and analyses

Variation in content of phenols among provenances

For each provenance, the average contents of magnolol, honokiol, total phenols (the sum of that of magnolol and honokiol) of *M. officinalis*, the ratio of magnolol to honokiol and their variations within the provenances are listed in Table 1. From the trial plantation, the best provenance came from Wufeng in the western part of Hubei, and the worst provenance from the Lushan Mountain of Jiangxi. The contents of honokiol had an obvious change among the provenances, ranging from 0.23% to 3.87%, and the highest provenance from Wufeng in the western part of Hubei was 16.83 times as much as the lowest provenance from the Lushan Mountain of Jiangxi. The total contents of phenols of provenances of Wufeng, Hefeng and Enshi in the western part of Hubei Province were the highest, and their average value (7.54%) was 5.06 times as much as that inferior provenance of the Lushan Mountain, and was 2.48 times as much as the two controls (3.04%) of Jingning, Zhejiang. The ratio of magnolol to honokiol of the provenances in the western part of Hubei was lowest (1.03-1.57), followed by that of Ziyuan, Guangxi (3.00), and other provenances ranged from 5.33 to 8.38, thus showed a good conformity on the whole. Variations in these four traits were in good conformity with the results directly obtained from the producing areas (Si *et al.* 1998). Therefore it can be said that differences in quality of *M. officinalis* bark produced in traditionally producing areas are not decided by the influence of environmental factors, such as local climate and soil, but resulted from genetic divergence of the *M. officinalis* population caused by long-term natural selection.

With regard to the variation among individuals of *M. officinalis* within a provenance, the variation of the contents of magnolol, honokiol and the total phenols were similar to those among provenances (see Table 1). The result showed that selection of individual trees on the basis of selection of provenance is not only of necessity but also of great potential in genetic improvement.

Table 1. Phenol contents and their variations among the provenances

Number of provenance	Name of provenance	Magnolol (%)	Honokiol (%)	Total phenols (%)	Magnolol / honokiol	Variation range in magnolol (%)	Variation range in honokiol (%)	Variation range total phenols (%)
1	Lushan, Jiangxi	1.26	0.23	1.49	5.48	0.70~2.08	0.11~.044	0.81~2.52
2	Shuichang, Zhejiang	2.60	0.34	2.94	7.65	1.26~3.92	0.14~0.51	1.40~4.37
3	Jingning1, Zhejiang	2.98	0.43	3.41	6.93	1.43~5.39	0.13~0.84	1.66~5.89
4	Jingning2, Zhejiang	2.36	0.30	2.66	7.87	0.88~6.73	0.18~0.62	1.10~7.35
5	Tiantai, Zhejiang	2.19	0.28	2.49	7.82	1.16~4.42	0.17~0.44	1.33~4.86
6	Guanxian, Sichuan	2.18	0.26	2.44	8.38	0.82~3.74	0.02~0.39	0.92~4.13
7	Pucheng, Fujian	2.40	0.31	2.71	7.74	1.06~5.56	0.10~0.74	1.17~6.06
8	Guangze, Fujian	2.91	0.35	3.26	8.31	1.39~4.74	0.20~0.56	1.60~5.15
9	Chengbu, Hunan	2.13	0.40	2.53	5.33	0.65~3.76	0.09~1.05	1.08~4.53
10	Ziyuan, Guangxi	2.91	0.97	3.88	3.00	0.54~5.28	0.31~2.35	1.14~7.63
11	Enshi, Hubei	3.84	3.56	7.40	1.08	1.30~7.87	1.22~5.28	2.52~13.15
12	Wufeng, Hubei	4.00	3.87	7.87	1.03	1.83~6.96	1.67~6.02	3.51~12.88
13	Hefeng, Hubei	4.50	2.86	7.36	1.57	2.32~7.94	0.91~5.23	4.02~12.37
	Average value	2.81	1.08	3.89	5.55	0.54~7.94	0.02~6.02	0.81~13.15

In terms of the total content of phenols in the 7-year-old *M. officinalis* plantation, the provenances from the western part of Hubei was higher than the content of the individuals of the same age in the original producing place (Si *et al.* 1998), and was more 2% than prescribed by the pharmacopoeia. As a result, the establishment of a plantation with superior provenances may shorten a production cycle to some extent, change management pattern, and enhance economic effects per unit of time.

The variance analysis for the traits was shown in Table 2. The result showed that four quality traits have a significant difference among the provenances, while no significant difference among replications. The bark thickness, tree height and DBH also had a significant difference among the provenances.

Table 2. Variance analysis of various traits of the provenances of *M. officinalis*

Source of variation	Degree of freedom	Traits	Mean square	F value	Traits	Mean square	F value
Provenance	12		2.2836	6.03^{**}		1763.2	3.58^{**}
Replication	2	Magnolol	0.1738		Tree height	7006.1	
Error	24		0.3785			0.1793	
Provenance	12		5.3595	77.70^{**}		492.1	2.56^{*}
Replication	2	Honokiol	0.0362		DBH	1.0742	
Error	24		0.0690			0.0701	
Provenance	12		13.6357	22.47^{**}		0.1220	3.11^{**}
Replication	2	Total phenols	0.3644		Bark thickness	0.1364	3.48^{**}
Error	24		0.6068			0.0392	
Provenance	12		25.2478	16.55^{**}			
Replication	2	Ratio of magnolol to honokiol	3.9491				
Error	24		1.5259				

Note: $F_{0.05}(12,24)=2.18$, $F_{0.01}(12,24)=3.03$; $F_{0.05}(2,24)=3.40$, $F_{0.01}(2,24)=5.61$

Heredity of the traits and their correlations among the provenances

Phenotypic and genetic correlations of the traits of leaf shape, oiliness, DBH, tree height, and bark thickness were analyzed (See Table 3).

The leaf shape of *M. officinalis* were divided into 3 types: convex-sharp in tip, slightly concave to level, and concave to deeply concave (Si *et al.* 1998). The analyzing results showed that leaf shape was significantly correlated with the contents of magnolo, honokiol, and the total phenols, with a high genetic correlation coefficient (0.7853-0.8407). The deeper the concave in the tip of a leaf, the lower the content. This means that the provenance from the western part of Hubei, which was convex in the tip of the leaf, had a high content of phenols. Moreover, leaf shape had a high heritability. Therefore, selection based on leaf shape was effective. The oiliness of *M. officinalis* bark was generally considered in correlation with tree age. The oily bark occurs when *M. officinalis* trees reach a certain age. The provenance from Wufeng and Hefeng in the western part of Hubei had oily bark and a high content of phenols, which indicates a high heritability regarding the oiliness of the bark (the provenance had a heritability of 0.9167). Oiliness of the bark was significantly correlated with the content of phenols, with a genetic correlation coefficient between 0.8841-0.8966.

Table 3. Phenotypic and genetic correlations of different traits of *M. officinalis* as well as heritabilities of provenance

Trait	Leaf shape	Oiliness	DBH	Tree height	Bark thickness	Magnolol	Honokiol	Total phenols	Magnolol/ honokiol
Leaf shape		0.6407			-0.6856	0.8407	0.7853	0.8204	-0.5070
Oiliness	0.5679^{*}				0.0713	0.8841	0.8939	0.8966	-0.6684
DBH	-0.4982	-0.1057							
Tree height	-0.3942	-0.0547	0.8196^{**}						
Bark thickness	-0.4411	0.2204	0.6911^{**}	0.5624^{*}		-0.3960	-0.2545	-0.3278	-0.0841
Magnolol	0.6014^{*}	0.7401^{**}	-0.5807^{*}	-0.6217^{*}	0.0816		0.9638	0.9908	-0.8409
Honokiol	0.6833^{**}	0.7628^{**}	-0.6705^{*}	-0.7112^{**}	0.0639	0.8533^{**}		0.9910	-0.9259
Total phenols	0.6640^{*}	0.7788^{**}	-0.6349^{*}	-0.6691^{**}	0.0764	0.9688^{**}	0.9546^{**}		-0.8919
Magnolol/honokiol	-0.4690	-0.5352	0.5763^{*}	0.7796^{**}	-0.1321	-0.4759	-0.7503^{**}	-0.6244^{*}	
Heritability	1.00	0.9167	0.7209	0.6088	0.6788	0.8342	0.9871	0.9555	0.9396

Note: In the upper triangle are listed genetic correlation coefficients; the lower triangle are listed phenotypic correlation coefficients; $R_{0.05}(11)=0.5529$, $R_{0.01}(11)=0.6835$; both tree height and DBH were estimated by the average of 3 replications.=

Both DBH and tree height were in significant correlation with the contents of different phenols, with a negative correlation with the content of phenols and a positive correlation with the ratio of the two phenols. With regard to the averages of tree height and DBH of different provenances, the provenances from the western part of Hubei were less than that of the provenances from Zhejiang. From a standpoint of population, the provenances from the western part of Hubei, which was convex-sharp in the tip of the leaf, and superior in quality, but didn't grow in Jingning of Zhejiang as well as in its original place. Bark thickness, differing from both tree height and DBH, had a low correlation coefficient in either phenotype or heredity. Analyses of correlation showed that selection regarding fast growth based on tree height and DBH would lead to decrease in quality. Because quality is most important to medicinal plants, the priority should go to quality first, followed by fast growing traits such as bark thickness, tree height, and DBH. Various quality traits were significantly correlated with each other in light of phenotype, with a genetic correlation coefficient between 0.8409 and 0.9910 as well as the heritability between 0.8342 and 0.9871. As compared with growth traits, quality traits had a high heritability. Therefore improvement in *M. officinalis* through quality traits is more effective than through growth traits. It can be predicted that work on the improvement of quality of *M. officinalis* should be both effective and beneficial.

Variation pattern for the content of phenols

Four traits of phenols and linear correlation coefficients for both latitude and longitude of seed collecting sites in different provenances were estimated separately (see Table 4). The results showed that the content of honokiol varied with longitude and latitude, with variations of longitude being dominant. From northwest to southeast, the content of honokiol decreased gradually, and that of the provenances from the western part of Hubei in the northeast were highest, which was convex-sharp in the tip of the leaf. Magnolol was different from honokiol and tended to decrease from the west to the east, only in negative correlation with longitude to some extent. The total content of phenols was similar to that of honokiol, while the ratio of the two phenols mainly varied with longitude, increasing from the east to the west. The analyzing result of the samples from the provenance plantation was similar to those of the samples directly from the producing areas.

Table 4. Correlation of the content of phenols of *M. officinalis* with both longitude and latitude of the producing areas

Sample source	Item	Magnolol	Honokiol	Total content of phenols	Magnolol/honokiol
Samples from the producing areas	Longitude	0.0166	-0.3832**	-0.1396	0.7142**
	Latitude	0.0591	0.2913*	0.1539	-0.3686**
Samples from the provenance plantation	Longitude	-0.6526*	-0.7356**	-0.7111**	0.7527**
	Latitude	0.5438	0.6969**	0.6287*	-0.5147

Note: $r_{0.05}(68)=0.2319$, $r_{0.01}(68)=0.3017$; $R_{0.05}(11)=0.5529$, $R_{0.01}(11)=0.6835$

Selection of superior provenances and individual trees

According to variations among the provenance and among individual trees within a provenance, the results of variance analyses and multiple comparisons showed that the 3 provenances of Wufeng, Enshi and Hefeng of Hubei had a higher content of phenols than other provenances, and higher than control by 142.11%-158.88%, the honokiol content of that was 6.73-9.46 times as much as that of control. The difference between the 3 provenances and the poorest provenance was even significant. Therefore they can be applied as superior provenances to extension (see Table 5).

Table 5. Comparison among superior provenances in the content of phenols of *M. officinalis*

Provenance	Average content (%)			Increment over control (%)			Increment over the poorest provenance (%)		
	Magnolol	Honokiol	Total phenols	Magnolol	Honokiol	Total phenols	Magnolol	Honokiol	Total phenols
Wufeng, Hubei	4.00	3.87	7.87	49.81	945.95	158.88	217.46	1582.61	428.19
Enshi, Hubei	3.84	3.56	7.40	43.82	862.16	143.42	204.76	1447.83	396.64
Hefeng, Hubei	4.50	2.86	7.36	68.54	672.97	142.11	257.14	1143.48	393.96
Jingning, Zhejiang	2.67	0.37	3.04				111.90	60.87	104.03
Lushan, Jiangxi	1.26	0.23	1.49						
Average	2.81	1.08	3.89	5.24	191.89	27.96	123.02	369.57	161.07

Using the method above, totally 10 individual trees was selected within the provenances, which had a total content of phenols between 9.89 and 13.15% (see Table 6). It can be seen that all these 10 superior individual trees came from superior provenances and their total content of phenols was 2.25-3.33 times as high as local provenance. There is a great potential for improvement.

Table 6. Comparison among superior individual trees in the content of phenols of *M. officinalis*

Provenance/ individual	Average content (%)			Increment over control (%)		
	Magnolol	Honokiol	Total phenols	Magnolol	Honokiol	Total phenols
Feng 53	5.93	5.59	11.52	122.09	1410.81	278.95
Feng 54	6.96	5.93	12.88	160.67	1502.70	323.68
Feng 71	6.19	5.54	11.73	131.84	1397.30	285.86
Feng 161	5.87	6.02	11.89	119.85	1527.03	291.12
Wufeng, Hubei	4.00	3.87	7.87	49.81	945.95	158.88
En 139	5.44	4.45	9.89	103.74	1102.70	225.33
En 140	7.87	5.28	13.15	194.76	1327.03	332.57
Enshi, Hubei	3.84	3.56	7.40	43.82	862.16	143.42
He 89	6.58	5.23	11.81	146.44	1313.51	288.49
He 91	7.15	3.25	10.40	167.79	778.38	242.11
He 177	5.94	4.40	10.34	122.47	1089.19	240.13
He 179	7.94	4.43	12.37	197.38	1097.30	306.91
Hefeng, Hubei	4.50	2.86	7.36	68.54	672.97	142.11
Jingning, Zhejiang (CK)	2.67	0.37	3.04			
Average of Provenance	2.81	1.08	3.89	5.24	191.89	27.96

Discussion

Great importance has been attached to the genuineness of *M. officinalis*, a traditional Chinese medicine. That means that where a medicinal plant comes from is a very important factor when quality is assessed. The result indicated that the producing area had an important influence on quality of *M. officinalis*, and the environment factor of producing areas, such as climate and soil, have led to the genetic divergence of the *M. officinalis* population. Thus this caused the occurrences of the geographical provenance and the superior individual trees, which are different from provenance and individual trees in heredity. All these lay a foundation for making use of superior provenance to select superior individual trees further with a view to increasing both quality and stability of *M. officinalis* bark.

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